

radiotherapy in human cancer models in vitro and in mouse xenografts.

**Conclusion:** Conclusion: GRP78 is a molecular target for the development of novel radiation sensitizing agents. Anti-GRP78 antibodies enhance the efficacy of radiotherapy when administered IV to mouse models of human cancer.

Poster: Radiobiology track: Tumour biology and microenvironment

#### PO-0986

**MiR-143 inhibits tumour progression by targeting STAT3 in esophageal squamous cell carcinoma**

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**Purpose or Objective:** The objective of this study was to investigate the biological role of miR-143 in esophageal squamous cell carcinoma (ESCC) progression and its underlying mechanism.

**Material and Methods:** Surgical tumor tissue samples were obtained from 40 patients. MiR-143 and STAT3 protein expression levels in these clinical samples and three ESCC cell lines were determined by quantitative RT-PCR and western blot. The relationship between expression level of miR-143 and clinical parameters were explored by one-way ANOVA. The specific targeting site of miR-143 in the 3'-UTR of STAT3 was identified using dual-luciferase reporter assays. Then, the effects of ectopic miR-143 or STAT3 expression on proliferation, cell cycle distribution, migration and invasion were determined in colony-forming assay, flow cytometry and transwell assay. The effect of miR-432 on tumor progression in vivo was determined by performing tumor formation assay in nude mice. The role miR-143 in regulating cell cycle signaling, epithelial-mesenchymal transition and MMP up-regulation through repressing STAT3 was explored by analyzing the expression level of the downstream proteins.

**Results:** MiR-143 expression was downregulated in 90% of the ESCC clinical samples and its expression level was associated with the lymph node metastasis(LNM), invasion and TNM stage in ESCC patients. Functional experiments showed that ectopic expression of miR-143 could inhibit tumor cell proliferation, migration and invasion by suppressing STAT3 in vitro. Animal experiments showed that the size of subcutaneous tumors derived from miR-143 overexpressing cells were significantly smaller than that of empty vector expressing cells. Further studies verified that miR-143 might regulate cell cycle, EMT and MMP up-regulation by targeting STAT3 and hence, lead to the suppression of ESCC cell proliferation, migration and invasion.

**Conclusion:** Our study showed that miR-143 could act as a tumor suppressor through the inhibition of proliferation, migration and invasion by directly targeting STAT3 and subsequently mediates the downstream proteins. Thus, miR-143 has significant value in clinical and may serve as a prognostic marker and therapeutic target in the future.

#### PO-0987

**MiR-432 inhibits tumor progression by targeting IGSF3 in esophageal squamous cell carcinoma**

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**Purpose or Objective:** The objective of this study was to investigate the biological role of miR-432 in esophageal squamous cell carcinoma (ESCC) progression and its underlying mechanism.

**Material and Methods:** Surgical tumor specimens and adjacent tissue samples were obtained from 40 patients. Pearson correlation coefficients and linear regression model were used to explore the bivariate correlations between miR-432 and IGSF3 expression levels and one-way ANOVA were used to estimate the relationship between expression level of miR-432 and clinical parameters. Then the effects of ectopic miR-432 or IGSF3 expression on proliferation and apoptosis were determined using miR-432 over-expression or knockdown cells in colony-forming assay and flow cytometry, and the effects on cell migration and invasion were determined using a transwell assay. On the other hand, bioinformatic analysis were performed to assess the relationship between IGSF3 and miR-432, and this relationship was identified using a dual-luciferase reporter assay. Finally, the biological consequences of miR-432-mediated suppression of IGSF3 expression in ESCC cell lines were also determined by performing colony-forming assay, flow cytometry and transwell assay.

**Results:** MiR-432 expression was downregulated in 93% (37/40) of the ESCC clinical samples and its expression level was associated with LNM and TNM stage in ESCC patients. Functional experiments showed that over-expression of miR-432 induced an inhibition of cell proliferation, promotion of apoptosis and suppression of cell migration and invasion in vitro by targeting IGSF3.

**Conclusion:** In conclusion, our results established a functional link between miR-432 and IGSF3 expression in esophageal cancer, demonstrating that IGSF3 was directly repressed by miR-432, which subsequently effects the tumor biological process. Collectively, this finding not only helped us understand the molecular mechanism of esophageal carcinogenesis, but also gave us a strong rationale to further investigate miR-432 as a potential biomarker and therapeutic target for esophageal cancer.

#### PO-0988

**Combined treatment strategies for microtubule interfering agent-resistant tumors**

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**Purpose or Objective:** Tumor cells are the major targets for classic anticancer treatment modalities. At the same time other cell types within the tumor microenvironment are also targeted and co-determine the treatment response. Resistances to specific treatment modalities are therefore not only linked to the mutated genetic background of the tumor cells but also to the interaction of tumor cells with the tumor microenvironment. Thus targeting of important elements of the microenvironment is a promising strategy to overcome treatment resistances in solid tumors. Here we mechanistically investigate in different clinically relevant microtubule-stabilizing agent (MSA)-refractory tumor models the potency of combined treatment modalities of MSAs, inhibitors of angiogenesis and ionizing radiation to overcome MSA-resistance.

**Material and Methods:** Rationally designed single and combined treatment regimens of ionizing radiation, microtubule stabilizing (taxane, epothilone) and destabilizing agents and anti-angiogenics compounds were investigated in genetically defined MSA-sensitive and MSA-resistant lung and colon adenocarcinoma cell lines in vitro and in the corresponding tumor xenografts in vivo.

**Results:** While MSAs potentially inhibited A549wt and endothelial cell proliferation, no anti-proliferative effect was observed in the corresponding mutated MSA-resistant tumor cells. Importantly, MSAs did not block anymore pro-survival auto- and paracrine signaling from resistant tumor cells by downregulation of HIF1-alpha transcriptional activity and subsequent secretions of HIF-1alpha-mediated growth factors and cytokines like VEGF. Thereby continuous pro-survival

signaling from these resistant tumor cells resulted in an additional level of treatment resistance towards the combined treatment modality of MSAs and ionizing radiation in vivo. However, combined treatment of MSAs with clinically relevant mTOR-signaling- or VEGF-antagonists strongly re-sensitized MSA-resistant tumors (lung and colon carcinoma models) to the corresponding MSA. Interestingly, a novel clinically relevant microtubule-destabilizing agent, which is still active in MSA-resistant tumors, successfully overcame MSA-resistance in the lung and colon carcinoma models, downregulated the HIF1- $\alpha$  related aggressive tumor phenotype and strongly sensitized for ionizing radiation (bolus and metronomic scheduling).

**Conclusion:** These data demonstrate that the interaction between the tumor cell compartment and the tumor microenvironment strongly determines the tumor response to the combined treatment modality of ionizing radiation and microtubule interfering agents. A combined treatment modality of microtubule interfering agents with antiangiogenic agents is potent to overcome tumor cell-linked MSA-resistance and should be considered as clinical strategy for MSA-refractory tumor entities alone and in combination with radiotherapy.

#### PO-0989

##### Hypoxic and perfusion effects of Trastuzumab in a HER2+ oesophageal adenocarcinoma xenograft model

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**Purpose or Objective:** We aimed to evaluate the pathological hypoxic and perfusion effects of Trastuzumab (T) and/or Cisplatin (C) in HER2+ oesophageal adenocarcinoma xenograft (OE19) which may potentially direct future clinical adjunctive therapy.

**Material and Methods:** SCID mice (n=17) bearing subcutaneous OE19 tumours were treated with either (i) Cisplatin 4mg/kg once a week, (ii) Trastuzumab 20mg/kg twice a week or (iii) Cisplatin and Trastuzumab for 2 weeks. Intraperitoneal Pimonidazole (Pm), an exogenous hypoxic marker, and intravenous Hoechst 33342 (Ho), a perfusion marker, were injected 2 hours and 1 minute prior to tumour excision, respectively. Tumours were immediately snap-frozen and 10 $\mu$ m frozen sections were obtained for immunofluorescence study. Following fixation, non-specific binding was blocked using 10% normal goat serum. The sections were then incubated overnight at 4°C with primary Pimonidazole FITC labelled mouse monoclonal antibody at 1:25 concentration. Propidium iodide (PI) was used as a counterstain to highlight morphology. Tumour sections were scanned using different filters for Pm (green), Ho (blue) and PI (red) on a fluorescence microscope at x100 magnification (Figure 1).

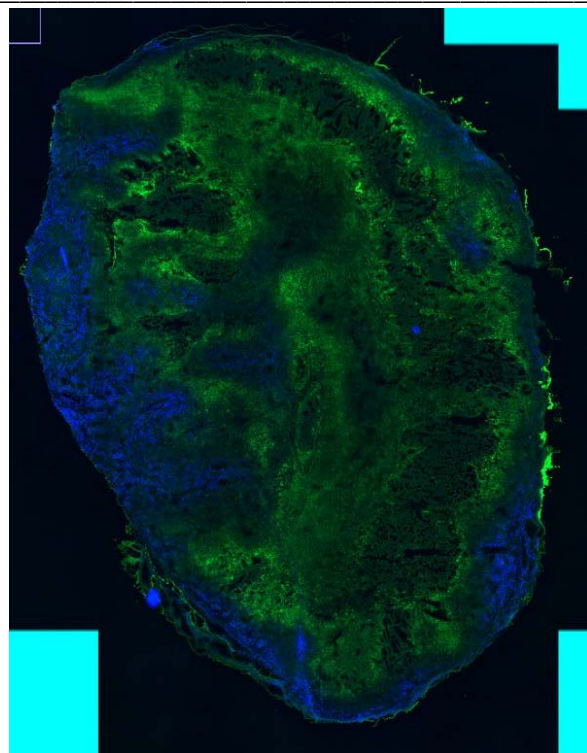


Image analysis was performed using the ImageJ software. Percentage areas stained with Pm (hypoxic fraction/HF) and Ho (perfusion fraction/PF) were derived and mean (%)  $\pm$  SD are presented. Difference in the HF and PF between Trastuzumab (T) and non-Trastuzumab (NT) treated animals were analysed.

**Results:** Overall, tumour periphery was better perfused in most tumours but there was no consistent hypoxic intratumoral spatial localisation. There was an inverse spatial relationship between Pm and Ho fluorescence in 10/17 tumours, colocalisation in 3/17 and no relationship found in 4 tumours. Trastuzumab-treated tumours (HF 38% $\pm$ 17) were less hypoxic compared to the NT group (HF 50% $\pm$ 13) and these tumours were also better perfused (PF: T 46% $\pm$ 25, NT 39% $\pm$ 16). Cisplatin-treated tumours had the highest HF (50% $\pm$ 13) and lowest PF (39% $\pm$ 16) compared to Trastuzumab (HF 34% $\pm$ 13, PF 48% $\pm$ 26) and combination therapy (HF 41% $\pm$ 21, PF 45% $\pm$ 27).

**Conclusion:** Trastuzumab appeared to exert the predominant proangiogenic effect with improved perfusion and reduced intratumoral hypoxia, although these effects were diminished with combination therapy. These data suggest that the addition of hypoxia-modifying agents might be tested as an adjunctive therapy, particularly in those not eligible or fit for Trastuzumab therapy.

Poster: Radiobiology track: Normal tissue effects: pathogenesis and treatment

#### PO-0990

##### Impact of Ramipril on rat spinal cord after high- and low-LET irradiation

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